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Discrimination of the aroma fraction of Sherry wines obtained by oxidative and biological ageing

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Abstract

Aroma compounds of fino, oloroso and amontillado Sherry wines, obtained by biological, oxidative and combined ageing, were analyzed. An analysis of variance was carried out for each compound, to classify the wines into different homogeneous groups. The compounds distinguishing the wines in the same way, and exceeding their perception thresholds, were subjected to discriminant analyses. This defined the groups of compounds more markedly influenced by the ageing, as well as their sensorial contribution to the flavour. As a consequence, the contents of several compounds present in the amontillado wines and their contribution to the odour profiles could be attributed to prevalence of their changes during the biological or oxidative ageing. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The Montilla-Moriles and Jerez regions (southern Spain) are prominent producers of so-called fino, amontillado and oloroso white wines. These three types of wine are produced by long ageing (5–12 years) in oak casks, starting from a must obtained from a single grape variety (cv. *Pedro Ximenez*), under identical fermentation conditions, but using different ageing procedures. As a result, the wines show differences in the contents of aroma compounds and endow them with peculiar sensory features.

Fino wines result from biological ageing, carried out by veil yeasts growing on the wine surface when the ethanol content is lower than 15.5% v/v. The aerobic metabolism developed by this type of yeast causes some changes in the aroma fraction that endow the wine with its typical flavour (Cortes, Moreno, Zea, Moyano, & Medina, 1998; Garcia-Maiquez, 1988; Martinez, Perez, & Benitez, 1997). Likewise, the veil yeasts protect against browning, allowing the wine to retain its pale colour for years (Baron, Mayen, Merida, & Medina, 1997).

Oloroso wines are obtained by oxidative ageing, after addition of ethanol up to a content about 18% v/v, which prevents the growth of veil yeasts (Botella, Perez-Rodriguez, Domecq, & Valpuesta, 1990; Casas, 1985; Domecq, 1989). Under these conditions, oloroso wine develops a dark colour as a result of the oxidation of phenolic compounds, distinguishing it clearly from fino wine (Fabios, Lopez-Toledano, Mayen, Merida, & Medina, 2000).

Finally, amontillado wines are obtained by ageing in a two-step process involving biological ageing under similar conditions to those of fino wines, followed by an increase in the ethanol content, they are then subjected to oxidative ageing, as in oloroso wines. Amontillado wines are thus the oldest and most valued of the three types, because they develop a more complex flavour than the other two. More detailed information about these ageing processes can be found in papers by Casas (1985), Domecq (1989) and Zea, Cortes, Moreno, and Medina (1996).

The purpose of this work was to compare the aroma fractions of Sherry wines of the fino and oloroso types, in order to identify differences in the typical aroma compounds ascribable to the ageing procedure used (biological or oxidative), as well as to observe the contribution of each stage to the composition of the aroma fraction of amontillado wines.

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2. Materials and methods

2.1. Wines

Nine samples of Sherry wines obtained by biological and oxidative ageing were selected from the Montilla-Moriles region (southern Spain). Triplicates of three samples were collected from wines that were biologically aged for 5 years under veil yeasts. Three other samples were also obtained in triplicate from wines exclusively subjected to oxidative ageing for 7 years (oloroso type wines). Finally, triplicates of three samples were collected from wines subjected to an initial biological ageing, similarly to the fino wines, followed by a second oxidative ageing step, such as that used for oloroso wines (amontillado wines). Because of their different productions, the three fino samples were selected by expert tasters as more representative among 78 of these wines, and the oloroso and amontillado samples were among 26 and 18, respectively. The ethanol contents in the fino, amontillado, and oloroso wines were 15.0±0.15, 19.0±0.76 and 18.7±0.30% v/v, respectively.

2.2. Experimental analyses

Acetaldehyde was quantified by an enzymatic test from Boehringer-Mannheim (Germany). For determination of the aroma compounds, samples of 100 ml of wine were adjusted to pH 3.5; 150 μg of 2-octanol was added as an internal standard and then extracted with 100 ml of freon-11 in a continuous extractor for 24 h. The compounds were quantified by GC (Hewlett-Packard 5890 series II) in a SP-1000 capillary column of 60 m×0.32 mm ID (Supelco Inc., Bellefonte, PA, USA) after concentration of the freon extracts to 0.2 ml. Three microlitres were injected into the chromatograph, equipped with a split/splitless injector and a FID detector. The oven temperature program was as follows: 5 min at 45°C, 1°C per min up to 195°C and 90 min at 195°C. Injector and detector temperatures were 275°C. The carrier gas was helium at 9 psi and split 1:100. The quantification was made by using chromatographic correction factors, calculated for each compound in relation to the internal standard in standard solutions of commercial products supplied by Sigma Aldrich (Germany). All the compounds were identified in previous laboratory works by means of Mass Spectrometry (Hewlett-Packard 5972 MSD).

2.3. Statistical procedures

A one-way analysis of variance was carried out on the replicated samples for each compound quantified in relation to three types of wine. The aroma compounds exceeding their perception threshold, and classifying the wines in different homogeneous groups, were subjected to discriminant analysis on the replicated samples. The computer program used was the Statgraphics™ (STSC Inc., Rockville, MD, USA).

3. Results and discussion

Table 1 lists the contents of aroma compounds in the three types of wine studied, the homogeneous groups of wines obtained by analysis of variance ($P<0.05$) for each compound, and their perception thresholds.

Fino wines, which are exclusively produced by biological ageing, can be distinguished from oloroso and amontillado wines, which are obtained by entirely or partly oxidative ageing, respectively, on the basis of the compounds that isolate the former in a group different from that formed by the latter two (a, b, b in Table 1). Such compounds were E- and Z-3-hexenol, ethyl isobutanoate, ethyl laurate, diethyl succinate, butanoic acid, 3-methylbutanoic acid, hexanoic acid, γ-butyrolactone, pantolactone, γ-decalactone, terpinen-4-ol, α-terpineol, Z-nerolidol, farnesol and octanal. As a rule, these compounds were present at increased levels in fino wines relative to the other two wine types, which suggests that they were produced in higher concentrations by the veil yeasts involved in the biological ageing process. Exceptions were ethyl laurate and diethyl succinate which were both at higher concentrations in the wines obtained by oxidative ageing, predictably because their synthesis in this ageing type must be favoured by a chemical pathway, as pointed out by authors such as Williams (1989).

In order to better observe the contribution of the compounds to the differentiation among the wines, the results for those that were present at concentrations higher than perception threshold, in at least one wine type, were subjected to a discriminant analysis. Table 2 lists the standardized coefficients for the two functions obtained by discriminant analysis carried out on the compounds distinguishing the wines in the groups a, b, b. The first function accumulated more than 99% of the discrimination, butanoic acid, farnesol and γ-decalactone being the compounds making the most marked contribution. In analytical terms, higher concentrations of these compounds distinguish the fino wine from the wines subjected to oxidative ageing. However, it is well known that each compound does not contribute to aroma according to its concentration, but through its perceived intensity. To estimate the sensory contribution of each compound to the overall flavour, the odour activity value (OAV) was calculated by dividing its concentration by its odour threshold (Table 2). Taking into account these OAVs, butanoic acid, farnesol, 3-methylbutanoic acid and γ-decalactone were the greatest contributing compounds to the flavour of fino wines. In addition, these compounds had higher concentrations than in the oloroso and amontillado wines; thereby the

Table 1
Aroma compound contents (mg/l) in the fino, amontillado and oloroso Sherry type wines, homogeneous groups ($P<0.05$), and odour threshold (mg/l)

Aroma compound	Fino type wine	Amontillado type wine	Oloroso type wine	Groups	Odor threshold
Acetaldehyde	545±44.7	183±65.8	126±19.5	a,b,c	100[a]
1,1-Diethoxyethane	58.8±13.1	19.9±10.5	50.2±15.2	a,b,a	1.0[a]
Acetoin	14.2±3.07	8.70±1.52	0.011±0.002	a,b,c	150[a]
Isobutanol	34.4±6.85	25.7±4.62	39.2±9.73	a,b,a	75[a]
Isoamyl alcohols	257±48.1	171±23.9	324±47.6	a,b,c	60[a]
Phenethyl alcohol	38.6±11.2	29.2±5.69	38.1±4.65	a,b,a	200[a]
2-Butanol	2.37±1.25	1.35±0.445	4.40±2.70	a,a,b	50[b]
1-Butanol	4.33±1.14	3.08±0.198	9.92±2.84	a,a,b	150[a]
1-Pentanol	0.088±0.016	0.057±0.017	0.116±0.020	a,b,c	64[a]
2-Methyl-1-pentanol	0.021±0.016	0.044±0.013	0.092±0.018	a,b,c	1.0[b]
4-Methyl-1-pentanol	0.029±0.022	nd[c]	0.135±0.062	a,a,b	1.0[b]
3-Methyl-1-pentanol	0.178±0.055	0.156±0.029	0.110±0.026	a,a,b	1.0[b]
1-Hexanol	0.895±0.059	0.754±0.128	1.41±0.186	a,b,c	1.1[a]
E-3-Hexenol	0.055±0.008	nd	nd	a,b,b	1.0[b]
Z-3-Hexenol	0.096±0.023	nd	nd	a,b,b	1.0[b]
1-Heptanol	nd	0.873±0.249	0.302±0.087	a,b,c	2.5[a]
1-Decanol	0.124±0.025	0.182±0.034	1.26±0.55	a,a,b	0.40[a]
Benzyl alcohol	1.77±0.287	0.573±0.265	3.30±1.84	a,b,c	900[a]
Methyl acetate	nd	nd	6.60±2.34	a,a,b	470[a]
Propyl acetate	0.059±0.012	0.162±0.018	0.088±0.033	a,b,c	4.7[a]
Isobutyl acetate	nd	nd	0.131±0.041	a,a,b	1.6[a]
Butyl acetate	nd	nd	0.161±0.039	a,a,b	1.8[a]
Ethyl acetate	13.9±5.51	46.5±9.45	183±53.4	a,b,c	12[a]
Ethyl propanoate	1.19±0.827	0.590±0.194	1.92±0.528	a,b,c	1.8[a]
Ethyl isobutanoate	1.19±0.181	0.548±0.118	0.436±0.091	a,b,b	5.0[a]
Ethyl butanoate	0.488±0.125	nd	0.577±0.156	a,b,a	0.40[a]
Ethyl heptanoate	0.078±0.010	0.109±0.024	0.021±0.035	a,b,c	0.22[a]
Ethyl octanoate	0.062±0.020	0.050±0.012	nd	a,a,b	0.58[a]
Ethyl decanoate	nd	nd	0.220±0.028	a,a,b	0.50[a]
Ethyl laurate	0.062±0.020	0.140±0.024	0.139±0.076	a,b,b	0.50[b]
Ethyl myristate	0.099±0.019	0.119±0.031	nd	a,a,b	0.50[b]
Ethyl palmitate	0.042±0.033	0.070±0.018	nd	a,b,c	1.0[b]
Ethyl pyruvate	0.162±0.097	0.342±0.075	1.55±0.211	a,b,c	5.0[b]
Ethyl benzoate	0.180±0.120	nd	0.215±0.060	a,b,a	0.50[b]
Diethyl succinate	25.4±4.47	52.7±11.4	55.4±13.4	a,b,b	1200[a]
Diethyl malate	9.00±4.05	7.78±1.49	23.6±6.10	a,a,b	10[b]
Ethyl lactate	108±5.68	184±56.3	307±79.5	a,b,c	150[a]
Methyl butanoate	nd	nd	0.486±0.269	a,a,b	0.10[b]
Isobutyl isobutanoate	nd	0.066±0.011	nd	a,b,a	1.0[b]
Propyl butanoate	0.112±0.063	0.150±0.032	nd	a,a,b	0.10[b]
Isobutyl lactate	0.034±0.010	nd	0.242±0.069	a,a,b	100[b]
Hexyl hexanoate	nd	nd	0.247±0.130	a,a,b	0.50[b]
Hexyl lactate	nd	nd	1.10±0.680	a,a,b	5.0[b]
Isoamyl laurate	nd	0.357±0.076	nd	a,b,a	100[b]
Phenethyl octanoate	0.190±0.109	0.275±0.089	nd	a,b,c	500[b]
Isobutanoic acid	nd	0.138±0.032	4.58±1.69	a,a,b	30[a]
Butanoic acid	14.6±6.11	0.607±0.231	1.31±0.807	a,b,b	2.2[a]
3-Methylbutanoic acid	6.79±3.46	1.82±1.39	nd	a,b,b	1.5[a]
Hexanoic acid	2.39±1.02	0.635±0.146	0.948±0.245	a,b,b	8.0[a]
γ-butyrolactone	40.8±9.60	30.4±6.64	28.0±9.24	a,b,b	20[d]
Pantolactone	5.22±2.04	3.12±2.04	1.70±0.581	a,b,b	2.0[e]
γ-decalactone	0.043±0.006	nd	nd	a,b,b	0.01[b]
Terpinen-4-ol	0.777±0.245	nd	nd	a,b,b	5.0[b]
α-terpineol	0.015±0.013	nd	nd	a,b,b	1.0[b]
β-citronellol	1.33±0.535	0.903±0.200	nd	a,b,c	0.10[a]
Nerol	0.151±0.066	0.176±0.052	nd	a,a,b	0.50[b]
β-ionone	0.062±0.017	0.045±0.023	nd	a,b,c	0.005[a]
E-nerolidol	0.076±0.031	0.213±0.063	0.120±0.039	a,b,a	1.0[b]
Z-nerolidol	0.696±0.104	nd	nd	a,b,b	1.0[b]
Farnesol	5.79±2.93	0.282±0.109	1.04±0.469	a,b,b	1.0[b]

(*continued on next page*)

Table 1 (*continued*)

Aroma compound	Fino type wine	Amontillado type wine	Oloroso type wine	Groups	Odor threshold
Methyleugenol	nd	nd	0.157±0.071	a,a,b	10[b]
Ethylguaiacol	0.106±0.062	0.137±0.066	0.224±0.080	a,a,b	0.02[a]
Eugenol	0.477±0.303	0.440±0.094	0.091±0.028	a,a,b	0.01[a]
p-Ethylphenol	nd	nd	0.094±0.010	a,a,b	140[a]
Octanal	0.090±0.011	nd	nd	a,b,b	0.05[b]
Furfural	0.179±0.129	3.23±0.595	7.34±2.86	a,b,c	150[a]
Methionol	0.063±0.021	0.354±0.140	0.894±0.573	a,a,b	1.5[a]

[a] From Etievant (1991).

[b] Determined by authors.

[c] nd, not detected.

[d] From Silva (1998).

[e] From Cutzach, Chatonnet, Henry, Pons, and Dubourdieu (1998).

Table 2
Discriminant functions coefficients, odour descriptors of aroma compounds classified in the a, b, b group exceeding the perception threshold, and odour activity values for fino wines

Aroma compound	Function 1 coefficient	Function 2 coefficient	Odour activity	Odour descriptors
Butanoic acid	−3.4313	−2.0864	6.66±2.78	Sharp, cheesy, rancid
3-Methylbutanoic acid	−1.6716	2.8036	4.53±2.30	Rancid, cheesy, sweaty
γ-Butyrolactone	−0.8294	−0.1566	2.04±0.48	Faint, sweet, caramel
Pantolactone	−0.4341	1.4462	2.61±1.02	Coconut, fatty
γ-Decalactone	2.1429	−0.4762	4.29±0.59	Fruity, peach, caramel
Farnesol	2.6197	−1.5907	5.80±2.93	Delicate, floral, oily
Octanal	1.8745	0.6665	1.80±0.23	Fatty, citrus

fino wines more strongly exhibit both positive notes (delicate, sweet, floral, fruity) and negative ones (cheese or rancid) than the other two. Currently the possible contribution to the flavour of several compounds, with individual contents lower than their threshold is not known. However, some compounds close to their threshold might contribute to fortify certain flavour notes. In fino wines, Z-nerolidol and ethyl isobutanoate could enhance floral and fruity notes, while hexanoic acid could contribute to the cheese and rancid notes.

On the other hand, the contents of 2-butanol, 1-butanol, 4-methyl-1-pentanol, 3-methyl-1-pentanol, 1-decanol, methyl acetate, isobutyl acetate, butyl acetate, ethyl octanoate, ethyl decanoate, ethyl myristate, diethyl malate, methyl butanoate, propyl butanoate, isobutyl lactate, hexyl hexanoate, hexyl lactate, isobutanoic acid, nerol, methyleugenol, ethylguaiacol, eugenol, *p*-ethylphenol and methionol distinguished oloroso wines, which undergo exclusively oxidative ageing, from fino and amontillado wines (a, a, b in Table 1), which are obtained exclusively or partly by biological ageing. As a rule, the contents of these compounds were higher in oloroso wines, because of their preferential production by chemical pathways during oxidative ageing and/or consumption by veil yeasts during biological ageing.

The discriminant analysis, carried out on the contents of the above compounds exceeding their perception threshold, provided two discriminant functions, the first of which accumulated 96.31% of the overall discrimination. Based on the standardized coefficients of this function (Table 3), 1-decanol, propyl butanoate and methyl butanoate were the compounds most clearly distinguishing the aroma fraction of oloroso wines in analytical terms. This is because of 1-decanol and methyl butanoate, which were at higher concentrations in these wines, while propyl butanoate was not detected in them. In sensorial terms, ethylguaiacol and eugenol showed OAVs much higher than the remaining compounds, although the concentration of the latter in the oloroso wine was lower than in the other two. Thereby more marked smoky notes are distinctive of the aroma of these wines. In relation to sub-thresholds, ethyl pyruvate, hexyl hexanoate, hexyl lactate and ethyl decanoate, exhibiting fruity notes, were the compounds with concentrations closer to their perception thresholds.

The wines obtained using both types of ageing (amontillado wines) can be distinguished from the other

Table 3
Discriminant functions coefficients, odour descriptors of aroma compounds classified in the a, a, b group exceeding the perception threshold, and odour activity values for oloroso wines

Aroma compunds	Function 1	Function 2 coefficient	Odour activity	Odour descriptors
1-Decanol	1.3899	1.8645	3.16±1.38	Floral, fruity, waxy
Diethyl malate	−0.4204	−0.1474	2.36±0.61	Fruity
Methyl butanoate	−0.4652	−1.6481	4.86±2.69	Fragrant, sweet
Propyl butanoate	−0.8345	0.5996	–	Sharp, pungent, rancid
Ethylguaiacol	0.0566	0.2410	11.2±3.99	Bacon, smoky
Eugenol	−0.1562	−0.4166	9.14±2.75	Strong, spicy, cinnamon

Table 4
Discriminant functions coefficients, odour descriptors of aroma compounds classified in the a, b, a group exceeding the perception threshold, and odour activity values for amontillado wines

Aroma compound	Function 1 coefficient	Function 2 coefficient	Odour activity	Odour descriptors
1,1-Diethoxyethane	0.4048	0.9230	19.9±10.5	Strong, tart, fruity
Ethyl butanoate	0.8653	−0.5168	–	Fruity, fragrant, sweet

Table 5
Discriminant functions coefficients, odour descriptors of aroma compounds classified in the a, b, c group exceeding the perception threshold, and odor activity values for the type of wine showing the highest value

Aroma	Function 1 coefficient	Function 2 coefficient	Odour activity	Odour descriptors
Acetaldehyde	1.2273	0.6675	5.45±0.45[a]	Ethereal, pungent
Isoamyl alcohols	−0.5847	0.5936	5.40±0.79[b]	Fusel oil, whisky
1-Hexanol	−0.6664	0.2595	1.28±0.17[b]	Herbaceous, woody, fragrant
Ethyl acetate	−0.9383	0.3888	15.2±4.45[b]	Pineapple, balsamic, ethereal
Ethyl propanoate	−0.2212	0.1468	1.07±0.29[b]	Sweet, ethereal, fruity
Ethyl lactate	1.2449	−0.5511	2.04±0.53[b]	Fruity, buttery, butterscotch
β-citronellol	−0.2183	−0.7232	13.3±5.35[a]	Green, citrus, fresh
β-ionone	0.8823	0.0138	12.4±3.37[a]	Balsamic, woody, warm

[a] Calculated for fino type wine.
[b] Calculated for oloroso type wine.

two on the basis of their contents of 1,1-diethoxyethane, isobutanol, phenethyl alcohol, ethyl butanoate, ethyl benzoate, isobutyl isobutanoate, isoamyl laurate and E-nerolidol (a, b, a in Table 1). The discriminant analysis performed on the compounds exceeding their perception threshold provided a function 1 that accumulated 97.15% of the overall discrimination (Table 4). Ethyl butanoate was not detected in amontillado wines and 1,1-diethoxythane was at a lower concentration in this type of wine than in the other two. Consequently, the OAV of the former cannot be calculated and the corresponding value of the latter was higher in fino and oloroso wines, so fruity notes are weaker in amontillado wines.

Finally, compounds such as acetaldehyde, acetoin, isoamyl alcohols, 1-pentanol, 2-methyl-1-pentanol, 1-hexanol, 1-heptanol, benzyl alcohol, propyl acetate, ethyl acetate, ethyl propanoate, ethyl heptanoate, ethyl palmitate, ethyl pyruvate, ethyl lactate, phenethyl octanoate, β-citronellol, β-ionone and furfural, distinguished the wines studied into three different groups (a, b, c in Table 1). Table 5 lists the results of the discriminant analysis carried out as described. The OAVs were calculated for the wine showing the highest concentration for each compound. The first discriminant function accumulated 87.53% of the discrimination. Based on its standardized coefficients, ethyl lactate, acetaldehyde and ethyl acetate were the compounds better-discriminating the three types of wine studied, in terms of concentration. However, the compounds contributing most to the flavour of fino wines were acetaldehyde, β-citronellol and β-ionone because of their higher OAVs in these wines. Enzymatic production of acetaldehyde by veil yeasts in wines obtained by biological ageing, is typical of this process (Casas, 1985; Garcia-Maiquez, 1988; Martinez et al., 1997), so fino wine exhibited the highest contents of this compound, which endows it with its typical pungent aroma. In addition, β-citronellol and β-ionone contribute markedly to aroma in these wines, with green, citrus and balsamic notes.

On the other hand, during oxidative ageing, which is exclusively used to obtain oloroso and partly amontillado wines, some chemical esterifications are especially strong, because of the large amounts of ethanol present. In this sense, Martinez, Perez, and Caro (1987) and Williams (1989) point out an increase in the contents of ethyl acetate and ethyl lactate, respectively, during this ageing type. Besides, veil yeasts consume ethyl acetate (Cortes et al., 1998; Mauricio, Moreno, Valero, Zea, Medina, & Ortega, 1993), so the wines subjected exclusively to oxidative ageing showed the highest contents of this ester. For these reasons, and according to OAVs, oloroso wines exhibited an aroma with pineapple, balsamic and ethereal notes, mainly as a result of the ethyl acetate contribution.

Summarizing, in terms of concentration, the statistical analyses carried out on the aroma fraction of Sherry wines, of the fino, amontillado and oloroso types, classified several compounds according to the biological or oxidative wine ageing (those figuring in the Tables 2 and 3, respectively). Amontillado wines are produced by combining the two ageing types; therefore the contents of the compounds listed in Table 2 could indicate a dominant contribution of the oxidative ageing stage, because their levels were not significantly different from those of the oloroso wines. For the same reason, the contents of the compounds in Table 3 could be attributed to a higher contribution of the biological ageing stage. Finally, the compounds listed in Tables 4 and 5 showed particular changes for the amontillado wines. However, it should be pointed out that compounds such as acetaldehyde, ethyl acetate, ethyl lactate, β-citronellol and β-ionone showed intermediate concentrations between fino and oloroso wines, revealing the participation of both types of ageing. From a sensorial view, by using the calculated OAVs and odour descriptors, the three types of wines studied showed different odour profiles. Fino wines are marked by floral and fruity (farnesol, β-citronellol and β-ionone), cheesy and rancid (butanoic acid), and pungent (acetaldehyde) notes. Oloroso wines exhibit smoky and ethereal notes associated with ethylguaiacol and ethyl acetate, respectively. Finally, amontillado wines inherit flavour notes from both ageing processes, resulting in the more complex aroma of the three wines studied.

More research should be undertaken in order to better observe the contribution, of each type of ageing to the composition of the amontillado wine, mainly focusing the length of each in relation to wine quality.

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